

1377-Pos Board B328**FRET Measurements of cAMP Dynamics in HL1 Cells Support the Key Role of Constitutive AC Activity in Cardiac Pacemaking**

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Spontaneously beating HL1 cardiac cells have evolved as a very useful tool to study regulation of pacemaking cardiac activity because of the ability to knock down specific molecules without generating KO mice in vivo. HL1 cells, however, have not been fully characterized and their pacemaker mechanisms may differ from those in cardiac pacemaking cells derived from adult heart. Basal constitutive adenylate cyclase activity is an obligate factor for normal spontaneous firing in adult sinoatrial nodal pacemaker cells. We expressed FRET sensors in spontaneously beating HL1 cardiac cells to measure cAMP concentration. The PDE inhibitor IBMX (100 μ M) increased the basal concentration of cAMP more than twofold and increased beating frequency by 70%. AC1 knockdown with siRNA completely inhibited the IBMX-induced increase in cAMP production in cytoplasm and plasma membrane, and decreased the beating frequency of HL1 cells. Pharmacological inhibition of both plasma membrane AC (2'-5' dideoxyadenosine) and cytoplasmic AC (KH7) selective blockers completely abolished the IBMX induced increase of cAMP concentration and resulted in complete cessation of spontaneous beating in HL1 cardiac cells. Thus, as in adult sinoatrial nodal pacemaker cells, basal AC activity is required for the spontaneous beating of HL1 cardiac cells.

1378-Pos Board B329**Abnormalities in Transmural Ventricular Electrophysiology in a Heterozygous SCN5A Knockout Mouse Model Revealed by Two-Photon Microscopy**

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The voltage-gated cardiac sodium channel Nav1.5, is a critical regulator of cardiac electrical excitability, responsible for the rapid upstroke of the action potential (AP) in both atrial and ventricular cardiomyocytes. In addition to regulating single cell electrophysiology Nav1.5 plays a critical role in conjunction with connexin43, in ventricular conductance. Mutations in the SCN5A gene encoding Nav1.5 are linked with several inherited arrhythmic disorders, including Brugada syndrome and long QT syndrome. Heterozygous mutant mouse models demonstrate severe conduction disturbances and intramural fibrosis, preferentially in the right ventricle (RV). Despite the observation that single cardiomyocyte upstroke velocity is significantly reduced, it has thus far been difficult to separate the relative contribution of reduced Nav1.5 conductance and intramural fibrosis to the appearance of lethal ventricular arrhythmias. We address this issue by characterizing in greater detail the transmural electrophysiological behavior of ventricular tissue in a mouse model of SCN5A downregulation. Heterozygous SCN5A^{+/-} mutant mice (SCN5A^{+/-}; n=7) and wild type (WT; n=6) littermates' hearts were Langendorff perfused beneath a combined 2P and epifluorescence microscope system and loaded with voltage (di-4 ANEPBS) and intracellular calcium (Fura-2AM) sensitive dyes. Under physiological endo-epicardial activation, no significant difference between groups was apparent from optical map recordings of the RV surface. Transmural 2P line scanning revealed significantly slower AP upstroke times within the subepicardium, but not close to the surface, in SCN5A^{+/-} vs WT. Conduction velocity was slower in SCN5A^{+/-} vs WT mice, and was preferentially slow in the midmyocardium vs epi/endocardium. Beat-to-beat activation was also significantly higher in SCN5A^{+/-} vs WT hearts. These data suggest significant conduction abnormalities within the midmyocardial wall which affect the rate and path of conduction preferentially within this transmural region.

1379-Pos Board B330**Features of Optical Mapping in Blue-Green and NIR Lights in Rabbit Heart**

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Optical signal is the image created from the propagating electrical signal. Due to optical features (absorption, scattering, etc.) of the heart tissue the coinci-

dence between optical and electrical action potentials (APs) is moderate. Having electrical action potentials recorded transmurally, we aimed to evaluate contribution of the electrical and the optical features on OAP upstroke formation using blue-green and near-infrared (NIR) lights. Here we introduce a new approach in analysis of the electrical activity from the optical mapping (OM) studies by recording transmural-APs with glass-microelectrodes and comparing them with the OAP, obtained using blue-green (di-4-ANEPPS) and NIR (di-4-ANBDQBS) dyes in Langendorff-perfused rabbit heart under atrial/endo-/epicardial pacing. We used averaged upstroke of transmural-APs for isolation of electrical and optical (of dye/tissue) impacts influencing formation of OAP upstroke shape, and this had helped to split the OAP upstroke into components (depth-weighted and lateral-scattering). These components separately reflect the transmural and the parallel to the epicardium electrical propagating wave. In addition, to calculate depth-weighted component, we used the probing-depth constant for fluorescence measurement that was detected directly during the OM experiment, but not from separate measurements of the excitation and the emission light penetration. The detected probing-depth constant (k) for the NIR dye was ~2 mm, while that magnitude was about twice smaller for the blue-green dye. The results of the study open a new opportunity for the future investigations of the electrical impulse propagation in the heart. This research was funded by the European Social Fund under the Global Grant measure.

1380-Pos Board B331**Comprehensive Analysis of Behavioral Variability in Real and Simulated Populations of Rabbit Left Ventricular Cardiomyocytes**

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Variability in behavior between cells and individuals is an important yet poorly studied phenomenon. Studies of cardiac physiology generally focus on typical behavior rather than quantitatively examining variability, and the causes and implications of behavioral variability in these systems are largely unknown. One reason for this is that the low-throughput nature of most physiology experiments makes studies of variability a daunting challenge. We implement two complementary approaches to overcome this: population-based mathematical modeling and higher-throughput experimental methods.

We have applied population-based modeling approaches to two models of electrophysiology and calcium handling in the rabbit LV cardiomyocyte, creating a population of cells by varying parameters representing levels of ion channels and transporters. This analysis reveals several key features of behavioral variability in these populations, including: (i) the same population shows much greater variability in calcium transient (CaT) amplitude than in action potential duration (APD), indicating a potentially fundamental propensity for variability in CaTs, (ii) covariation occurs between outputs (e.g. positive correlations between CaT duration and APD) and enables prediction of the effects of some perturbations (e.g. partial block of L-type current) based on baseline behaviors.

This theoretical analysis is complemented with experimental measurements of variability using the recently-developed CelloPTIQ system, which allows repeat optical measurements of APs and CaTs to be made with increased throughput (up to 100 cells in 2-3 hours). Early measurements of APD50 from cells isolated from the LV of a single rabbit (n=85 cells) show an approximately normal distribution (mean=297 ms, SD=46 ms, range 151-445 ms), with cells from other rabbits showing similar distributions. These experimental measurements allow for comprehensive quantification of variability and testing of hypotheses generated by model analysis (e.g. correlations between outputs and different degrees of variability between outputs).

1381-Pos Board B332**Application of the RIMARC Algorithm to a Large Data Set of Action Potentials and Clinical Parameters for Risk Prediction of Atrial Fibrillation**

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Ex-vivo recorded action potentials (APs) in human right atrial tissue from patients in sinus rhythm (SR) or atrial fibrillation (AF) display a characteristic